IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.	:	10/091,300
Applicants	:	Patricia Rockwell et al.
Filed	:	March 4, 2002
Art Unit.	:	1642
Examiner	:	David J. Blanchard
Docket No.	:	11245/46211

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

DECLARATION UNDER 37 C.F.R. 1.132

Dear Sir:

I, James R. Tonra, Ph.D, declare that:

I am the Associate Director of Experimental Therapeutics at ImClone Systems Incorporated.

The following study was undertaken under my supervision and direction:

A pancreatic adenocarcinoma line BxPC-3 was obtained from the American Type Culture Collection (Manassas, VA) and cultured at 37°C/5% CO2 in RPM1640 (Invitrogen Corporation, Carlsbad, CA) supplemented with 10% fetal bovine serum (HyClone, Lenexa, KY) and 1% GlutaMAX (Invitrogen Corporation). Cells were passaged or collected for injection using Trypsin EDTA (Invitrogen Corporation).

6-7 week old female athymic (nu/nu) mice were injected subcutaneously with 3×10^6 BxPC-3 pancreatic tumor cells in 50% culture media and 50% reconstituted basement membrane (MATRIGEL®) (BD Biosciences, San Jose, CA). When the mean tumor volume reached approximately 250 mm^3, mice were randomized by tumor volume into treatment groups. Treatment involved administering either an EGFR antibody (ERBITUX®) alone, a VEGFR antibody (DC101) alone, or a combination of an EGFR antibody and a VEGFR antibody. ERBITUX® is a chimeric (human/mouse) IgG monoclonal anti-EGFR antibody

(GenBank Accession No. 1NQLA) and DC101 is an anti-mouse flk-1 antibody (produced by hybridoma cell line deposited as ATCC HB 11534). Human IgG (BioDesign International, Saca, MA) and rat IgG (Equitech-Bio Inc., Kerryville, TX) were used as controls. The treatment groups were as follows, each treatment group consisting of 4-12 mice:

Treatment Group #	Treatment Concentrations	Treatment Schedule
	Erbitux/CD101 Combination Therapy	
1	5 mg/kg Erbitux + 40 mg DC101	
2	1.25 mg/kg Erbitux + 10 mg/kg DC101	
3	0.5 mg/kg Erbitux + 4 mg/kg DC101	
4	0.125 mg/kg Erbitux +1 mg/kg DC101	
5	0.05 mg/kg Erbitux + 0.4 mg/kg DC101	
	Erbitux Monotherapy	
6	5 mg/kg Erbitux	Tuesday-Friday
7	1.25 mg/kg Erbitux	Tuesday-Friday
8	0.5 mg/kg Erbitux	Tuesday-Friday
9	0.125 mg/kg Erbitux	Tuesday-Friday
10	0.05 mg/kg Erbitux	Tuesday-Friday
	DC101 Monotherapy	
11	40 mg/kg DC101	Monday-Wednesday-Friday
12	10 mg/kg DC101	Monday-Wednesday-Friday
13	4 mg/kg DC101	Monday-Wednesday-Friday
14	1 mg/kg DC101	Monday-Wednesday-Friday
15	0.4 mg/kg DC101	Monday-Wednesday-Friday
	Control	
16	Saline (10 ul/gram)	Monday-Wednesday-Friday
17	40 mg/kg rat IgG (M-W-F) + 5 mg/kg human IgG (M-Th)	Monday-Wednesday-Friday (rat IgG) Monday-Thursday (human IgG)

All antibodies were diluted in United States Pharmacopeia (USP) saline (Braun). Antibody doses were based on separate dose response studies to provide a dose response curve that would provide an accurate estimate of the dose resulting in an individual mouse T/C%=50 for each of the three treatment categories. Tumor volumes were recorded approximately twice a week for 33 days. Tumor volume was calculated as $\pi/2 * (L * W^2)$, where L is the longest diameter measured with caliphers, and W is the diameter perpendicular to L.

Statistics

The T/C% for each mouse was calculated, wherein T/C% =

$$100 * \frac{\text{(final individual experimental mouse tumor volume / initial individual experimental mouse tumor volume)}}{\text{(final mean control group mouse tumor volume / initial mean control group mouse tumor volume)}}$$

From each of the three categories of treatment, described above, a predicted dose causing a %T/C value of 50 (ED50) and a 96.61% confidence interval for ED50 was calculated using Fieller's method on the least squares linear regression of individual mouse T/C% versus $\log_{10}$(dose)(JMP version 5 software, Cary, North Carolina). The dose of the combination treatment is taken as the sum of the doses of each component. From the 96.61% confidence interval for $\log_{10}$[ED50], lower confidence limits for ERBITUX® ED50 and DC101 ED50, and an upper confidence limit for the combination group ED50 were obtained such that the probability of the ED50 being beyond these limits was α=1.695%. Using these three limits, an upper limit (UL) for the combination index (CI) was calculated (ULCI), wherein CI= A+B, wherein A is the dose of DC101 in the combination treatment at ED50 for the combination, divided by the ED50 for DC101 monotherapy; B is the dose of ERBITUX® in the combination treatment at ED50 for the combination, divided by the ED50 for ERBITUX® monotherapy. If CI<1, the treatments are considered synergistic. For CI to be >ULCI, one or more of the three ED50 limits would have to be breached. The probability of this occurring is $3\alpha-3\alpha^2+\alpha^3 = 5\%$, thus CI<ULCI with >95% confidence.

The frequency of regressions was analyzed using a Chi Squared test. For all test, $p<0.05$ was considered significant.

Results

Figure 1 illustrates the dose response curves for ERBITUX® monotherapy, DC101 monotherapy, and a combination of DC101:ERBITUX® at a 8:1 dose ratio. Since the saline and IgG control groups did not differ in terms of tumor growth, these two groups were pooled into a single group for the calculation of individual mouse T/C% in the treated groups. In both monotherapies and combination groups, a significant correlation was found between the $\log_{10}$[dose] and individual mouse T/C% ($p<0.02$ for all three). ED50 values were 16.60 mg/kg for DC101 and 1.78 mg/kg for ERBITUX®. For the combination groups, the ED50 was 0.86 mg/kg, indicating a dose of 0.10 mg/kg ERBITUX® and 0.76 mg/kg DC101. These values were used to calculate a CI = (0.1/1.78) + (0.76/16.6) =0.1, indicating synergism. Synergism indicates that the amount of each treatment in combination needed to achieve a certain effect (e.g. T/C% = 50%) is less than would be predicted by simply adding the effect expected from each monotherapy.

It is noted that if the two fractions used to calculate CI are multiplied by 100 they are in fact the percentage of each monotherapy ED50 in the combination treatment that results in an individual mouse T/C%=50. When there is no interaction between the treatments, these two percentages will add to 100 (CI=1), but when CI=0.1, the percentages add to 10. Thus CI=0.1 indicates that on average, 10% of the expected total dosages of the efficacious monotherapies are necessary to achieve the targeted efficacy, when these treatments are given in combination.

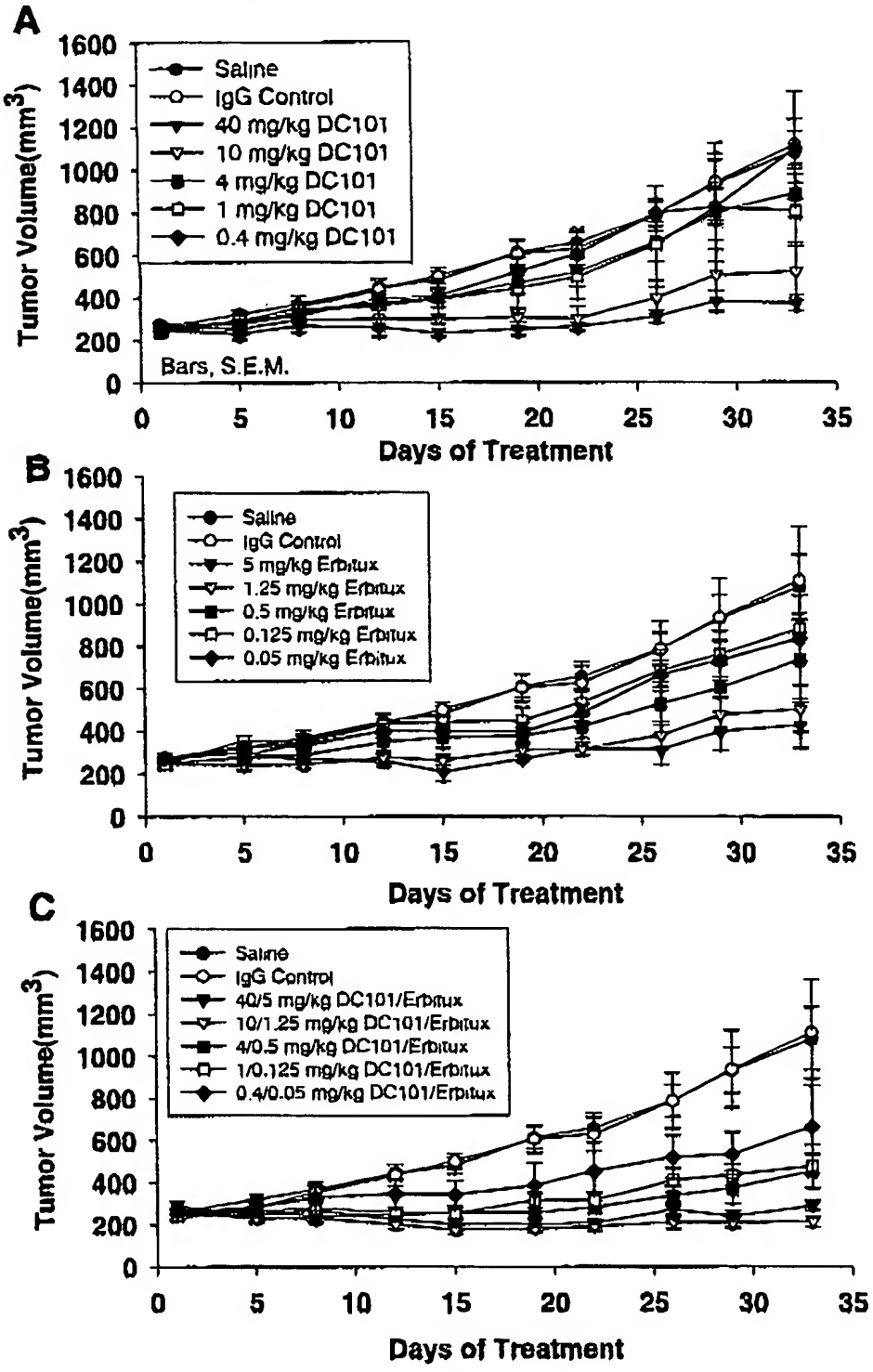
The lower confidence limit for the ED50 of DC101 from the 96.61% confidence limit calculated using Fieller's method for inverse prediction was 6.35 mg/kg. The lower confidence limit for the ED50 of ERBITUX® was 0.49 mg/kg. The upper confidence limit for the ED50 of the combination group was 2.56 mg/kg, indicating a dose of 0.28 mg/kg ERBITUX and 2.28 mg/kg of DC101. Using these values, a 95% upper confidence limit for CI (ULCI) is calculated. In this case, ULCI=0.93. For CI to be greater than 0.93, one or more of the confidence limits used to calculate ULCI would have to be breached. The chance

this happening is only 5%. Thus, DC101 and ERBITUX® are synergistic when dosed in combination at a 8:1 dose ratio, with greater than 95% confidence, and a predicted CI =0.1.

Conclusions

The effects of DC101 and ERBITUX® were synergistic when dosed in combination at an 8:1 dose ratio, with greater than 95% confidence and a predicted CI=0.1

FIGURE 1: Dose Responses in the BxPC-3 Xenograft Model



PATENT
Appl. No. 10/091,300
11245/46211

The undersigned declares that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title `8 of the United States Code and that willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signed this 29 day of September, 2005

Dr. James R. Tonra